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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

NON PEPTIDE AGONISTS AND ANTOGONISTS OF ADRENOMEDULLIN AND GASTRIC RELEASING PEPTIDE

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☒ Drawing(s) *Number of Sheets* 6 ☒ Other (specify) Appendix

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☐ Applicant claims small entity status. See 37 CFR 1.27.

X X A check or money order is enclosed to cover the filing fees — FILING FEE AMOUNT ($)

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U.S. Government agency is National Institutes of Health.

Respectfully submitted,

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PROVISIONAL APPLICATION FOR
UNITED STATES LETTERS PATENT

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INVENTION: NON PEPTIDE AGONISTS AND ANTOGONISTS OF ADRENOMEDULLIN AND GASTRIC RELEASING PEPTIDE

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NON PEPTIDE AGONISTS AND ANTAGONISTS OF ADRENOMEDULLIN AND GASTRIC RELEASING PEPTIDE

FIELD OF THE INVENTION

This invention relates generally to small molecule, non-peptide, modulators (*e.g.*, antagonists or agonists) of peptide hormones. Also described are complexes comprising such small molecules, methods of identifying the molecules as modulatory agents, and methods of diagnosis or treatment, using the molecules.

BACKGROUND INFORMATION

Adrenomedullin (AM) is a peptide hormone implicated in the pathophysiology of important diseases such as hypertension, cancer, and diabetes. AM is a 52 amino acid peptide that belongs to the calcitonin/calcitonin gene related peptide (CGRP)/amylin/AM superfamily. In humans, this peptide is expressed by many cell types and exerts a variety of physiological roles, including vasodilatation, bronchodilatation, regulation of hormone secretion, neurotransmission, antimicrobial activities, regulation of growth, apoptosis, migration, and angiogenesis, among others.

These activities are mediated by a complex receptor system encompassing a seven transmembrane domain polypeptide known as calcitonin receptor-like receptor (CRLR), a single transmembrane domain protein, termed receptor activity modifying protein (RAMP), and the intracellular receptor component protein (RCP). RCP is necessary for the initiation of the signal transduction pathway. Three RAMPs have been identified in mammals and their coexpression with CRLR results in different binding affinities, with RAMP1 producing a characteristic CGRP-1 response whereas coexpression of CRLR with RAMP2 or RAMP3 elicits a specific AM receptor.

Gastrin releasing hormone (GRP) is a peptide hormone implicated in the pathophysiology of important diseases such as cancer and respiratory problems in premature babies. GRP is a 27 amino acid peptide, initially identified as the human counterpart of bombesin, a peptide found in the frog's skin. GRP has a variety of physiological roles. For example, it has antimicrobial properties, reduces food intake, and has been involved in respiratory development, and in the regulation of short-term memory, among others.

Several types of antagonists have been proposed for peptide hormones, including monoclonal antibodies and inhibitory peptide fragments, such as AM(22-52), AM(16-31), AM(11-26), and proAM(153-185). While these molecules are effective as research tools, they sometimes exhibit significant limitations as pharmaceutical agents, *e.g.*, because of the lack of humanized blocking antibodies and the short biological half-life of fragmentary peptides. There is a need for additional agents that modulate activities of peptide hormones, in particular AM and GRP, and that can be used to treat disease conditions mediated by the peptide hormones, such as the conditions noted above. Small molecule, non-peptide agents would be particularly desirable.

DESCRIPTION OF THE INVENTION

This invention relates, *e.g.*, to agents, particularly small molecule, non-peptide, agents, that modulate activities of peptides that interact with specific receptors. In preferred embodiments, the peptides whose activities are modulated are peptide hormones, most preferably adrenomedullin (AM) or gastrin releasing peptide (GRP).

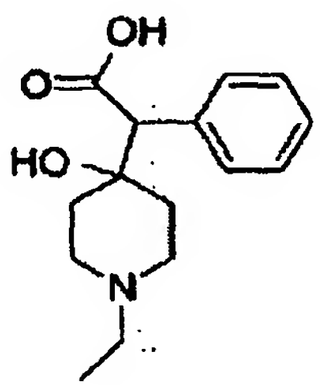
The present inventors have developed a two-step screening method to identify such modulatory agents. AM and GRP were used as exemplary peptide hormones in the screening assay; a variety of other peptide hormones can, of course, also be used. The term "modulate," as used herein, includes to increase, stimulate, augment, enhance, facilitate, or potentiate, or to decrease, inhibit, suppress, interfere with, prevent, block, etc. An agent that augments the activity of a peptide hormone is said to be an agonist (in some cases, as discussed below, a superagonist); an agent that suppresses the activity is said to be an antagonist. Both AM and GRP exhibit a variety of "activities," some of which are described elsewhere herein.

To identify modulatory compounds, a library of known small molecule, non-peptide, compounds was screened. Compounds were first identified on the basis of their ability to interfere with binding between AM or GRP and their respective blocking antibodies. Compounds identified as "positive" in this first step were further screened for their ability to influence receptor-mediated biological activities (inhibition of the induction of second messengers). Using this two-step procedure, seven compounds were identified as antagonists of AM, and three as antagonists of GRP. Surprisingly, in view

of the fact that the compounds were first identified because of their ability to *inhibit* the binding of the peptide to its blocking antibody, other compounds were identified that act as agonists (*e.g.*, superagonists) of the peptides. Six superagonists were identified for AM, and one for GRP. A total of 17 modulatory agents were identified.

Among the advantages of the identified small molecule, non-peptide, modulatory agents are that the molecules are stable, especially when in an organism; are small and thus exhibit good cell permeability characteristics; and are readily synthesized, allowing for the rapid, inexpensive production of large quantities.

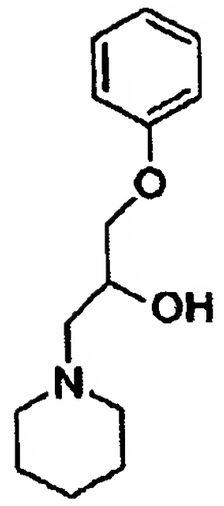
In one embodiment, the invention relates to a method for modulating an activity of an adrenomedullin (AM) peptide, comprising contacting the peptide with an effective amount of a compound of (structures of some of the following compounds are provided in the Appendix attached to this provisional application):



formula I,

formula II ,

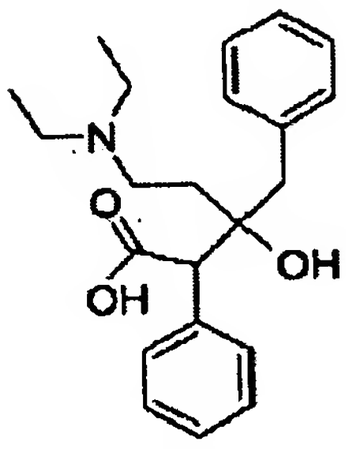
formula III,



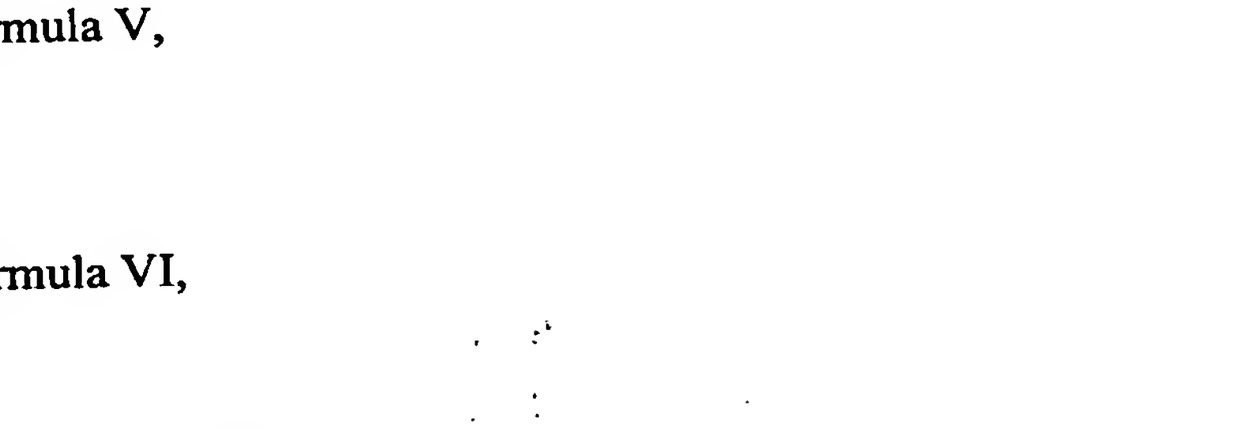
formula IV,

formula V,

formula VI,



formula VII,

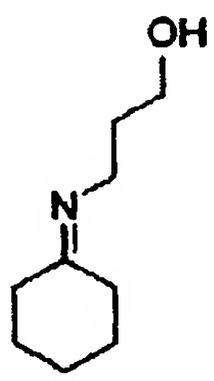


formula VIII,

formula IX,

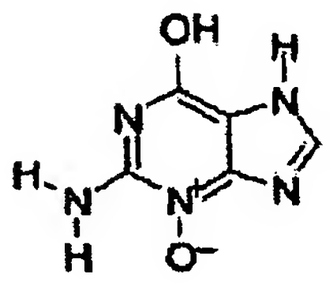
formula X,

formula XI,



formula XII,

or



formula XIII.

In one embodiment, the modulation is the inhibition of an AM peptide activity, and the compound is represented by one of formula I through formula VII. The activity that is inhibited may be, *e.g.*, stimulation of the level of intracellular cAMP, vasodilation, or the like. Another embodiment is a method for treating a condition that is mediated by over-expression and/or -activity of AM, comprising administering to a patient in need of such treatment an effective amount of a compound of formula I, II, III, IV, V, VI or VII. Among suitable conditions for such treatment are type 2 diabetes or cancer.

In another embodiment, the modulation is the stimulation of an AM peptide activity, and the compound is represented by one of formula VIII through formula XIII. The activity that is inhibited may be, *e.g.*, stimulating the level of intracellular cAMP,

vasodilation, or the like. Another embodiment is a method for treating a condition that is mediated by under-expression and/or -activity of AM, comprising administering to a patient in need of such treatment an effective amount of a compound of formula VIII, IX, X, XI, XII or XIII. Among suitable conditions for such treatment are renal or cardiovascular disese, sepsis, or central nervous system ischemia.

In embodiments of the preceding methods to inhibit or stimulate AM, the peptide and the compound are in an animal, such as a mammal (*e.g.*, following the administration of the compound to the animal *in vivo*), or the peptide and the compound are *in vitro* (not in an animal).

In another embodiment, the invention relates to a method for modulating an activity of a gastrin releasing peptide (GRP) peptide, comprising contacting the peptide with an effective amount of a compound of

formula XIV,

formula XV,

formula XVI, or

formula XVII.

In one embodiment, the modulation is the inhibition of a GRP peptide activity, and the compound is represented by one of formula XIV through formula XVI. The

activity that is inhibited may be, *e.g.*, stimulating intracellular levels of IP_3 or Ca^{+2}, or stimulating angiogenesis, suppressing food intake, regulating glucose homeostasis, or stimulating hypotension. Another embodiment is a method for treating a condition that is mediated by over-expression and/or -activity of GRP, comprising administering to a patient in need of such treatment an effective amount of a compound of formula XIV, XV or XVI. Among suitable treatment methods are reducing tumor growth, treating low blood pressure (hypotension) or an eating disorder (such as anorexia or bulimia).

In another embodiment, the modulation is the stimulation of a GRP peptide activity, and the compound is represented by formula XVII. The activity that is inhibited may be, *e.g.*, stimulating intracellular levels of IP_3 (inositol phosphate) or Ca^{+2}, or stimulating angiogenesis, suppressing food intake, regulating glucose homeostasis, or stimulating hypotension. Another embodiment is a method for treating a condition that is mediated by under-expression and/or -activity of GRP, and/or that would benefit from an increased expression or activity of a GRP activity (such as angiogenesis), comprising administering to a patient in need of such treatment an effective amount of a compound of formula XVII. Among suitable conditions for such treatment are obesity, diabetes or hypertension. Furthermore, the method may be a method for treating a condition in which stimulation of angiogenesis is desirable, *e.g.*, coronary or peripheral artery disease, tissue ischemia, organ or tissue transplantation, and acceleration or enhancing of fracture repair or wound healing.

In embodiments of the preceding methods to inhibit or stimulate GRP, the peptide and the compound are in an animal, such as a mammal (*e.g.*, following the administration of the compound to the animal *in vivo*), or the peptide and the compound are *in vitro* (not in an animal).

In another embodiment, the invention relates to a complex, comprising a compound of formula I through formula XIII, in association with (*e.g.*, bound to) an AM peptide, or comprising a compound of formula XIV through formula XVII, in association with (*e.g.*, bound to) a GRP peptide. The complex may be in an animal, such as a mammal (*e.g.*, following the administration of the compound to the animal *in vivo*), or it may be *in vitro* (not in an animal).

In another embodiment, the invention relates to a complex comprising a compound of formula I through formula XIII, in association with (*e.g.*, bound to) a blocking antibody of AM, or comprising a compound of formula XIV to formula XVII, in association with (*e.g.*, bound to) a blocking antibody of GRP. The complex may be in an animal, such as a mammal, or it may be *in vitro* (not in an animal).

In another embodiment, the invention relates to a composition comprising compound of formula I through formula XIII, in association with (*e.g.*, bound to) an AM peptide, or comprising a compound of formula XIV through formula XVII, in association with (*e.g.*, bound to) a GRP peptide. In another embodiment, the invention relates to a composition comprising a compound of formula XIV through formula XVII, in association with (*e.g.*, bound to) a GRP peptide. The compositions may be in an animal, such as a mammal (*e.g.*, following the administration of the compound to the animal *in vivo*), or they may be *in vitro* (not in an animal).

In another embodiment, the invention relates to a pharmaceutical composition, comprising a compound of formula I through formula XVII and a pharmaceutically acceptable carrier.

In another embodiment, the invention relates to a method (*e.g.*, a diagnostic method) for detecting an AM peptide, comprising contacting a sample suspected of containing the peptide with one or more detectably labeled compounds of formula I through formula XIII, and detecting labeled compound that is associated with (bound to) the peptide; or for detecting a GRP peptide, comprising contacting a sample suspected of containing the peptide with one or more detectably labeled compounds of formula XIV through Formula XVII, and detecting labeled compound that is associated with (bound to) the peptide. The detection method may be performed *in vivo* or *in vitro*. Optionally, for example when the detection is performed *in vivo*, the detectably labeled compound(s) may be in the form of a pharmaceutical composition.

In other embodiments, the invention relates to kits suitable for treating subjects in need of such treatment. In one embodiment, the kit is suitable for treating a subject suffering from a condition mediated by aberrant expression and/or activity of adrenomedullin (AM); and it comprises one or more of the compounds of formula I to formula XIII, or a pharmaceutical composition comprising said compound(s) and a

pharmaceutically acceptable carrier and, optionally, a packaging material. In another embodiment, the kit is suitable for treating a subject suffering from a condition mediated by aberrant expression and/or activity of gastrin releasing peptide (GRP); and it comprises one or more of the compounds of formula XIV to formula XVII, or a pharmaceutical composition comprising said compound(s) and a pharmaceutically acceptable carrier and, optionally, a packaging material.

In other embodiments, the invention relates to kits suitable for detecting an AM or GRP peptide (*e.g.*, for a diagnostic method). In one embodiment, the kit is suitable for detecting an AM peptide; and it comprises one or more of the compounds of formula I to formula XIII, which is detectably labeled, and, optionally, means to detect the labeled compound associated with (bound to) the peptide. In another embodiment, the kit is suitable for detecting a GRP peptide; and it comprises one or more of the compounds of formula XIV to formula XVII, which is detectably labeled, and, optionally, means detect the labeled compound associated with (bound to) the peptide. Kits suitable for *in vivo* detection may further comprise a pharmaceutically acceptable carrier.

As noted above, the inventors have developed a two-step screening method to identify agents which modulate an activity of, *e.g.*, a peptide hormone. As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, an agent of the invention that modulates "an" activity of a peptide hormone of interest may modulate one or more such activities.

In the experiments reported herein, modulatory agents were identified for AM and GRP.

As a starting point, molecules of the NCI small molecule (non-peptide) library were screened. This library contains about $5x10^6$ molecules, which are organized into 2,000 families grouped under the criterion of chemical similarity (Voigt *et al.* (2001) *J. Chem. Inf. Comput. Sci.* 41, 702-712). Structures of the compounds are available at the web site cactus.nci.nih.gov/ncidb2. Any library of small organic or inorganic molecules, such as molecules obtained from combinatorial and natural product libraries, can be tested and identified by the methods of the invention.

Furthermore, other types of molecules can also be screened, including (1) peptides, such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries, such as is described in Lam *et al.* (1991) *Nature* 354, 82-84; Houghten *et al.* (1991) *Nature* 354, 84-86), and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids, and specific derivatives of peptides of interest; (2) antibodies (*e.g.*, polyclonal, monoclonal, humanized, anti-idiotype, chimeric, and single chain antibodies as well as Fab, $F(ab')_2$, Fab expression library fragments, and epitope-binding fragments of antibodies); and (3) phosphopeptides, such as members of random and partially degenerate, directed phosphopeptide libraries, *e.g.*, as in Songyang *et al.* (1993) *Cell* 72, 767-778.

The method of the invention was designed based on the assumption that a neutralizing antibody, such as a monoclonal antibody, binds to an epitope on the peptide that is important, if not critical, for receptor recognition. Without wishing to be bound by any particular mechanism it appears that the inventors confirmed this assumption by the identification of biologically active compounds capable of modulating the physiology of AM and GRP. In addition, the antibody-based colorimetric screening procedure allows for high throughput formats able to analyze thousands of compounds (or more) in very short periods of time.

The first step in the procedure exemplified herein was to identify compounds that interfered with binding between the peptide and its blocking monoclonal antibody. Some details of this first step in the assay are presented in Example 3. See also the results shown in Fig. 1A. In many cases, active compounds could be identified by the naked eye, even before colorimetric quantification (see Fig. 1B). 2,000 parental compounds of the library were screened using this methodology for AM, and 121 of them caused a significant inhibition in color intensity in a statistically significant fashion.

The inventors also screened the same compounds with a blocking monoclonal antibody against GRP (Chaudhry *et al.* (1999) *Clin. Cancer Res.* 5, 3385-3393), a peptide similar to AM in size and in chemical characteristics. This allowed the evaluation of the specificity of this methodology, as well as the identification of modulatory agents for GRP. Screening the same clinical library, 109 compounds were identified that inhibited color formation to a significant degree. Only 5 of them were also present among the

molecules able to interfere with AM, indicating that, in fact, different combinations of peptide-antibody complexes pulled out distinct sets of small molecules. This clearly shows that this methodology is able to discriminate between target molecules

As is discussed below, many of the compounds identified in this first step of the assay were not useful for modulating receptor-mediated responses. In the experiments reported herein, only 19.8% of the compounds tested for AM, and 4.6% of the compounds tested for GRP, fulfilled this criterion. Nevertheless, this first step allowed for a rapid primary screening of a large number of compounds, and reduced considerably the number of compounds that must be tested with the more expensive and time-consuming cell-based screen.

Since the first step of the screening strategy was based on the ability of the test molecules to interfere with the binding between the peptide and its antibody, it was possible that not all these molecules would also modify the binding between the peptide and its receptor, even though the monoclonal antibodies used were shown to be neutralizing. To investigate functional consequences of the molecules identified in the first step of the screen, all the "positive" compounds were subjected to an analysis of their ability to modify the production of the intracellular second messenger elicited by the specific receptor system.

All the compounds chosen with the primary AM screening were analyzed with a cAMP assay. Details of this second step of the assay are present in Example 4a. From the initial 121 compounds, 24 were able to significantly modulate the amount of cAMP induced by 100 nM AM in Rat2 cells, whereas the other 97 did not modify the cAMP response to AM. Interestingly, some of these compounds reduced the cAMP levels (acted as antagonists) whereas others actually elevated intracellular cAMP levels over the levels induced by AM alone, identifying them as superagonists (Fig. 2A, Table 1). In the absence of AM, none of the compounds elicited any response (Fig. 2A), suggesting that the mechanism of action includes binding of the small molecule to AM rather than to the receptor. That is, the molecules acted as superagonists rather than as agonists. These responses were dose-dependent, with drug responses seen with chemical concentrations as low as 10 nM (Fig. 2B).

Peptide	Action on second messengers	Code[1]	Biological activity
AM	Antagonists	16311 (compound I)	Elevates blood pressure
		37133 (compound II)	
		48747 (compound III)	Elevates blood pressure
		89435 (compound IV)	Elevates blood pressure
		28086 (compound V)	
		79422 (compound VI)	
		50161 (compound VII)	
	Superagonists	697165 (compound VIII)	
		697162 (compound IX)	
		697168 (compound X)	
		697169 (compound XI)	
		128911 (compound XII)	Reduces blood pressure
		145425 (compound XIII)	Reduces blood pressure
GRP	Antagonists	54671 (compound XIV)	
		77427 (compound XV)	Inhibits cord formation

		112200 (compound XVI)	
	Superagonist	372874 (compound XVII)	

Table 1.

Table 1 shows compounds that induced consistent effects on modulating second messenger activation by AM or GRP. Some of them were tested for biological activity (4th column). [1] The chemical structures of these compounds can be found at the web site: cactus.nci.gov/ncidb2.

The primary difference between AM and CGRP receptors is the nature of the particular RAMP that is associated to CRLR. When the active compounds for AM were added to a CGRP receptor-containing cell in the presence of synthetic CGRP, no effect was observed (Fig. 2D), demonstrating the specificity of these compounds for the binding between AM and its receptor.

For the compounds that showed promising behavior by both screening steps, close structurally related chemical family members were also evaluated. The prediction was that a similar chemical structure would predict similar biological behavior. In most cases, this analysis produced compounds with stronger activity than the original substance (Fig. 2C), suggesting that grouping compounds based on their chemical similarity could be useful to predict their potential biological activity. That is, one could use a modular approach to the screening process, beginning with the leading 2,000 compounds and then with other members of the promising families. This strategy, combined with our high throughput antibody-based primary screening, allowed for a complete preliminary search of the whole library in a matter of days.

In a similar approach, the small molecules that were identified in the first screening step with the GRP antibody were characterized by their ability to modify IP_3 or Ca^{2+} levels induced by synthetic GRP in cells containing its receptor (see Example 4b and Fig. 3). Again, both antagonist and superagonist molecules were identified. As was the case with modulators of AM, the GRP-interfering small molecules by themselves did not produce any change in IP_3 levels (Fig. 3A). In the Ca^{2+} assay, 1 nM GRP produced a marked elevation of intracellular Ca^{2+} in H1299 cells (Fig. 3B), but pre-exposure of the

cells to the identified antagonists greatly reduced the Ca^{2+} spike amplitude (Fig. 3C). The compounds that showed a consistent behavior with either the AM or the GRP systems are summarized in Table 1.

To validate the biological activity of some of the small molecules selected above, several assays were performed, as is described in more details in Examples 5 and 6. For example, an important function of AM is the regulation of blood pressure. Injection of screen selected AM superagonists (at 20 nmols/Kg) in hypertensive rats induced a profound and long-lasting decrease from basal levels in blood pressure ranging from 50 to 70 mm Hg (Fig. 4A,B). Vehicle alone (DMSO in PBS) at the same concentration did not alter blood pressure (Fig. 4A). On the other hand, when screen selected small molecule AM antagonists were injected into normotensive animals, also at 20 nmols/Kg, an elevation in blood pressure was observed (Fig. 4C). The blood pressure profile generated by the superagonists was similar to the one elicited by the peptide itself (that was used as a control in Fig. 4B), suggesting that these small molecules may be enhancing the effect of circulating AM.

The inventors also analyzed the influence of some of the GRP antagonists in an angiogenic model. GRP (5 nM) was able to induce cord formation in a culture of endothelial cells grown on Matrigel (Fig. 5A,B) whereas the addition of 0.5 μM of the screen identified compound 77427 greatly reduced the complexity of the tubular lattice (Fig. 5C). The number of knots per photographic field went from 3 ± 1 (control) to 37 ± 5 for the addition of 5nM GRP ($p<0.001$) and back to 12 ± 4 when GRP and 77427 were added together (compared to control $p=0.02$, compared to GRP alone $p=0.003$).

In a preliminary analysis, the inventors have identified some elements that appear be conserved among the modulatory agents identified herein. Without wishing to be bound by any particular model, it is suggested that the modulatory agents fall into several "families" of structures. A careful analysis of the chemical structures of some of the active compounds for AM reveals some common characteristics (Fig. 6). The most active antagonists have in common an aromatic ring separated from a three-substituted nitrogen by 4 elements. There is also a hydroxy group at 2 or 3 elements from the nitrogen (Fig. 6A). The compounds with superagonist activity share the presence of nitrogenated heterocycles with oxygen atoms at similar distances (Fig. 6B).

Nevertheless, the surprising simplicity of compound compound XII (128911) suggests that the activity may be due just to the presence of a nitrogen with sp^2 hybridization situated at a determined distance from the oxygen.

Once the three-dimensional structures of the AM-AM receptor complex and the GRP-GRP receptor complexes have been resolved, it should allow one to identify the binding sites of the small molecules and to introduce direct design modifications of our molecules to fit the active site more closely. Such methods are conventional. See, *e.g.*, rational design methods in Ghosh *et al.* (2001) *Curr. Cancer Drug Targets* 1, 129-140. Additional optimization can be obtained by generating additional compounds by combinatorial chemistry, for example by modifying slightly the chemical backbone identified here with different radicals. Such methods are conventional. See, *e.g.*, Gray *et al.* (1998) *Science* 281, 533-538.

The invention relates to a method to identify an agent that modulates (*e.g.*, modulates an activity of) a peptide which interacts specifically with a receptor, such as a peptide hormone, preferably AM or GRP, comprising

a) contacting the peptide, a blocking antibody of the peptide, and a putative binding-inhibitory agent,

b) detecting binding of the peptide to the antibody, and

c) selecting an agent which inhibits (*e.g.*, disrupts) said binding, compared to the binding in the absence of the putative binding-inhibitory agent, thereby identifying a binding-inhibitory agent.

The binding-inhibitory agent may be an antagonist or an agonist of the peptide. Preferably, the putative binding-inhibitory agent is a non-peptide small molecule. In one embodiment, the method is a high throughput method (assay).

In a preferred embodiment, the above method further comprises

d) contacting a binding-inhibitory agent identified as above, the peptide, and a cell that comprises a receptor for the peptide,

e) detecting the amount in the cell of a second messenger induced by the peptide, and

f) selecting an agent that modulates the amount of the second messenger in the cell, compared to the amount in the cell in the absence of the agent, thereby identifying a modulatory agent.

The modulatory agent may be an antagonist or an agonist (*e.g.*, a superagonist) of the peptide. For example, the agent may be an agonist or antagonist of an activity of the peptide, such as its binding to a receptor, the stimulation (expression) of a second messenger, or any of the other activities described elsewhere herein. In one embodiment, the cell comprises a receptor for AM, and the second messenger is AM-induced cAMP. In another embodiment, the cell comprises a receptor for gastrin releasing hormone (GRP), and the second messenger is GRP-induced IP_3 or Ca^{++}. Preferably, the putative binding-inhibitory agent is a non-peptide small molecule. In one embodiment, the method is a high throughput method (assay).

Any of the preceding methods for identifying putative modulatory agents may further comprise additional steps, some of which are discussed elsewhere herein. The invention also relates to modulatory agents which are identified and/or characterized by a method of the invention, particularly small, non-peptide, molecules that are encompassed by one of the generic structures identified herein.

Modulatory agents of the invention (*e.g.*, small molecule non-peptide compounds) can be prepared (*e.g.*, synthesized) fully conventionally, using known reaction chemistry, starting from known materials or materials conventionally preparable. Procedures for synthesizing small molecule, non-peptide, compounds can readily produce gram amounts of a compound of interest. Many compounds of the invention are readily available from standard sources, such as chemical supply houses, or can be generated from commercially available compounds by routine modifications.

Agents of the invention may be used in therapeutic methods for conditions that are mediated by aberrant expression and/or activity of a peptide hormone, such as AM or GRP, and/or for conditions that respond to an increase or decrease in an activity of the peptide hormone. The term "aberrant" expression and/or activity, as used herein, includes expression or activity that is higher or lower than a base line value, such as the amount present in a subject who does not exhibit symptoms of the condition, or who does

not exhibit a predisposition to the condition. The expression or activity may be an "under" -expression or -activity, or an "over" -expression or -activity. When aberrant expression results in undesirable symptoms, the condition is sometimes said to be a pathological condition.

The therapeutic methods include diagnosis, treatment, prevention, and/or amelioration of symptoms of any of a variety of conditions (*e.g.*, pathological conditions) in a subject. The subject (*e.g.*, a patient) can be any suitable animal, *e.g.*, mammal, such as experimental animals (*e.g.*, mouse, rat, guinea pig, or rabbit); pets (*e.g.*, mammals, birds, reptiles, fish, amphibians); farm animals; and primates, especially humans.

With regard to agents that modulate AM activity, AM levels are dysregulated in many pathologies (*e.g.*, in humans), such as hypertension, heart failure, sepsis, cancer, or diabetes, when compared to healthy controls. This correlation, together with experimental actions of AM in relevant model systems, implicates this molecule in the pathophysiology of such conditions. Interestingly, changes in AM levels may have apparently paradoxical effects on a patient's health, depending on the particular disease studied.

For example, elevated AM expression seems to exert a protective role in renal and cardiovascular diseases, sepsis, and in central nervous system ischemia. Without wishing to be bound by any particular mechanism, it is suggested that overexpression of AM is protective due to its vasodilator activity. An agent that acts as an agonist or superagonist of AM can be used to treat or prevent conditions that are ameliorated by the expression of AM, such as, *e.g.*, vascular diseases, trauma, malignant hypotension, catecholamine disorders, or the other conditions noted above.

In other circumstances, elevated AM expression appears to worsen a pathological condition, such as the progression of type 2 diabetes and cancer. In diabetic rats, injection of AM results in a reduction of circulating insulin levels and a concomitant hyperglycemia, whereas application of a monoclonal antibody against AM lowers glucose levels and ameliorates postprandial hyperglycemia. AM antagonists of the invention may be used to treat diabetes, *e.g.*, by regulating insulin secretion and/or blood glucose metabolism. In cancer cells, AM acts as a tumor survival factor. This tumor survival may be influenced by various activities of AM, such as elevation of tumor cell

growth, circumventing apoptosis, increasing migration, and enhancement of angiogenesis. Among the types of neoplastic transformation (*e.g.*, cancerous cells) that can be treated by AM antagonists of the invention are, *e.g.*, adrenal, nervous system, lung, colon, ovarian and breast cancerous cells, and chondrosarcoma. An agent that acts as an antagonist of AM can be used to treat or prevent conditions that are rendered worse by the expression of AM, such as the conditions noted above, or others.

Additional conditions that can be diagnosed, treated, and/or prevented with antagonists or agonists (*e.g.*, superagonists) of AM will be evident to the skilled worker. Among the physiological effects of AM are bronchodilation, regulation of hormone secretion, neurotransmission, antimicrobial activities, and regulation of cell growth and migration. One of skill in the art will recognize a variety of conditions that are mediated by these, or other, effects. Among the treatment methods for which agents of the invention are suitable are, *e.g.*, treating conditions related to pregnancy (*e.g.*, diagnosing and/or treating preeclampsia or promoting fetal growth); regulating activity in areas of the central nervous system (*e.g.*, regulation of neurotransmission or neuron growth, such as in, *e.g.*, Alzheimer's disease); lessening or inhibiting the allergic response due to the degranulation of mast cells; treating bacterial and fungal infections by inhibiting or preventing bacterial or fungal growth; facilitating the healing of chafed skin, skin lesions, wound repair, and surgical incisions (*e.g.*, by applying to the surface of the skin of a subject an amount of one or more of the agents of the present invention effective to facilitate healing); and promoting organ and bone development.

For a further discussion of some conditions that can be diagnosed, treated and/or prevented with AM antagonists or agonists, and suitable methods that can be applied to use of the modulatory compounds of the invention, see U.S. patent application 20020055615 (Cuttitta *et al.*).

With regard to agents that modulate GRP activity, GRP levels are dysregulated in many pathologies (*e.g.*, in humans), such as cancers, compared to healthy controls. The inventors have used a neutralizing monoclonal antibody against GRP in phase I/II clinical trials of previously treated small cell lung cancer patients (Chaudhry *et al.* (1999) *Clin. Cancer Res.* 5, 3385-3393). The results of that trial, including a curative complete response, suggest that inhibitors of GRP biology may be very useful in addressing

clinical problems. In addition, GRP as well as other endocrine peptides have been shown to promote angiogenesis. Angiogenesis is a complex process that requires endothelial cell growth and migration, extracellular matrix remodeling, formation of tubular structures, and loop formation among other mechanisms. The studies reported herein show the ability of some small molecules to interfere with the cord formation ability of GRP. GRP by itself promoted the development of a complex meshwork made of pseudo-capillaries. The simultaneous application of compound XV (77427) resulted in a marked decrease in the complexity of the tubular network, indicating a utility of this compound (or others identified herein) in antiangiogenic interventions. In addition to the treatment or prevention of, *e.g.*, cancers (*e.g.*, reduction of tumor growth) with GRP antagonists, GRP agonists (*e.g.*, superagonists) are useful for treating conditions in which increased angiogenesis is desirable. Such conditions include, *e.g.*, coronary or peripheral artery disease; any form of tissue ischemia resulting from vascular occlusion, vascular disease or surgery (*e.g.*, peripheral limb ischemia or hepatic arterial occlusion in liver transplantation); organ or tissue transplantation (*e.g.*, liver organogenesis, or in conjunction with cellular therapy and transplantation of pancreatic islet cells in the treatment of diabetes, as vascular endothelium acts to stimulate or induce pancreatic organogenesis and insulin production by pancreatic beta cells); and acceleration or enhancing of fracture repair or wound healing (including recovery from surgical wounds).

GRP is involved in a number of other physiological functions, which will be evident to a skilled worker. These functions include, *e.g.*, the suppression of food intake, regulation of glucose homeostasis, and enhancement of hypotension. Among the conditions that can be treated or prevented with GRP antagonists are, *e.g.*, eating disorders (such as anorexia or bulimia, in which stimulation of food intake is desirable) and low blood pressure (hypotension). Among the conditions which can be treated or prevented with GRP agonists (*e.g.*, superagonsits) are, *e.g.*, obesity, diabetes or hypertension. Other conditions suitable for treatment with GRP antagonists or agonists will be evident to the skilled worker. For a discussion of some physiological functions of GRP, and some disease conditions that can be treated or prevented with antagonists or agonists of GRP, see, *e.g.*, Mantey *et al.* (2001) *The Journal of Biological Chemistry* 276,

9219-9229; Merali *et al.* (1999) *Neuropeptides* 33, 376-386; and Ohki-Hamazaki *et al.* (1997) *Nature* 390, 165-169.

Any of the suggested treatment or prevention methods using AM or GRP antagonists or agonists (*e.g.*, superagonists) may be combined with other therapeutic modalities, and combinations of the agents of the invention may be used.

In some of the inventive methods for modulating an activity of a peptide, or for detecting a peptide, the peptide is "contacted" with a modulatory agent of the invention. This contacting may be achieved in a subject (*in vivo*) or outside of an animal (*in vitro*). Suitable methods for contacting are conventional and well-known in the art. For example, a peptide can be contacted with a compound in a cell (either *in vivo* or *in vitro*) by introducing the compound by injection, such as microinjection, electroporation, sonoporation, a gene gun, liposome delivery (*e.g.*, Lipofectin®, Lipofectamine® (GIBCO-BRL, Inc., Gaithersburg, MD), Superfect® (Qiagen, Inc. Hilden, Germany) and Transfectam® (Promega Biotec, Inc., Madison, WI), or other liposomes developed according to procedures standard in the art), or receptor-mediated uptake and other endocytosis mechanisms.

In methods of treatment according to the invention, an effective amount of an agent of the invention is administered to a subject. The term "an effective amount," as used herein, means an amount that elicits a detectable response (*e.g.*, amelioration of a symptom or a physiological response); the degree of the response can be minimal, provided that it is detectable. Similarly, in methods for modulating an activity of a peptide, an effective amount of an agent of the invention is contacted with the peptide. An "effective amount" in this context means an amount that elicits a detectably amount of modulation.

In methods of treatment, the agent can be administered by any of a variety of conventional procedures. Suitable routes of administration include parenteral and non-parenteral routes. Parenteral routes include, *e.g.*, intravenous, intraarterial, intraportal, intramuscular, subcutaneous, intraperitoneal; intraspinal, intrathecal, intracerebroventricular, intracranial, intrapleural or other routes of injection. Non-parenteral routes include, *e.g.*, oral, nasal, transdermal, pulmonary, rectal, buccal, vaginal, ocular. In a preferred embodiment, the administration is timed, slow-release,

aerosolized administration. Administration may also be by continuous infusion, local administration, "directed systemic" administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection.

Dosages to be administered can be determined by conventional procedures known to those of skill in the art. See, *e.g.*, The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds., Macmillan Publishing Co., New York. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Factors to be considered include the activity of the specific therapeutic agent involved, its metabolic stability and length of action, mode and time of administration, drug combination, rate of excretion, the species being treated, and the age, body weight, general health, sex, diet, and severity of the particular disease-states of the host undergoing therapy. Dosages can be selected in a manner customary for treatment with comparable agents for the same condition.

The agents of the invention may be formulated as pharmaceutical compositions, with any of a variety of conventional, pharmaceutically acceptable carriers, diluents and/or excipients. For suitable components and methods of preparing pharmaceutical compositions, see, *e.g.*, Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company (1990); the Handbook of Pharmaceutical Excipients, American Pharmaceutical Association (current edition); and Pharmaceutical Dosage Forms: Tablets (Lieberman, Lachman and Schwartz, eds., current edition, published by Marcel Dekker, Inc.

Agents of the invention may also be used in detection (*e.g.*, diagnostic) procedures. For example, compounds of the invention can be labeled with conventional labels, using conventional procedures, and then used to detect AM or GRP, *in vivo* or *in vitro* (*ex vivo*). Compounds in which a detectable label is present are sometimes referred to herein as "detectably labeled" compounds. Methods (means) of detecting such detectable labels (*e.g.*, detecting labeled compounds that have become associated with (*e.g.*, bound to) the peptide) are conventional and well-established. The detection may be direct, or indirect (*e.g.*, as in some enzymatic detection methods). In some embodiments, the detection is quantitative.

With regard to *in vivo* imaging, since both AM and GRP are turned over rapidly in the body, there is little circulating AM or GRP. Thus, *in vivo* detection (imaging) to determine where AM or GRP is localized in an organism can indicate the site at which the peptide is produced. Labeled compounds of the invention can be used in any situation in which a tracer of AM or GRP is desirable. Suitable labels will be evident to a skilled worker and include, *e.g.*, heavy metals, which can be detected in PET scans, and radioactive labels, such as ^{131}I or other short-lived radioactive tracers. Because many cancers are associated with the production of large amounts of AM, detection (diagnostic) methods as above with AM modulatory compounds are useful for detecting the presence and/or location of a cancer. Other uses of such *in vivo* detection (diagnostic) methods will be evident to the skilled worker.

As for *in vitro* methods (assays), a compound of the invention can be labeled with a conventional detectable label, such as a fluor or an enzyme (*e.g.*, lactoperoxidase, alkaline phosphatase, or beta galactosidase), and then contacted with a tissue sample (such as a pathology sample) in order to visualize the presence of the peptide (*e.g.*, to identify a cancerous tissue). The compounds of the invention can be used in a variety of *in vitro* assay procedures, not only to detect the presence of a peptide of interest, but also to quantitate the amount of the peptide. For example, the compound can substitute for a monoclonal antibody in a conventional radioimmunoassay. In addition, the modulatory agents can be used for pharmacological drug design. For example, by analyzing the three dimensional structure of a complex between an AM or GRP peptide, or a blocking antibody for the peptide, and a compound identified herein or a variant thereof, using NMR or NMR imaging, one can screen and/or characterize variants that are more effective antagonists or agonists than the starting compound. (The compounds identified herein can serve as comparative controls in such a method.) Other suitable *in vitro* methods in which compounds of the invention can be used will be evident to the skilled worker.

Detection methods of the invention can be used to detect (*e.g.*, diagnose or monitor) any of the conditions described elsewhere herein, or others, which are mediated by aberrant expression and/or activity of AM or GRP. For example, one can monitor a condition (*e.g.*, a disease condition) by measuring the amount of AM or GRP in a sample,

wherein the presence of the AM or GRP indicates the existence of, or predisposition to, the condition. Examples of conditions that can be diagnosed or monitored by methods of the invention include, but are not limited to, diabetes; renal diseases, such as severe uremia; bone diseases, such as neoplastic disease; skin diseases; and blood related diseases, such as leukemia.

In another aspect of the invention, modulatory compounds of the invention are found in complexes with (or are in compositions with) the AM or GRP peptides, or with blocking antibodies specific for those peptides. In the complexes (or compositions) of the invention, the compounds associate with (*e.g.*, bind to) the peptides or antibodies by any of a variety of means that are well-know to skilled workers. The types of association include, *e.g.*, covalent bonds or non-covalent bonds (*e.g.*, passively adsorbed, such as by electrostatic forces, ionic or hydrogen bonds, hydrophilic or hydrophobic interactions, Van der Waals forces, etc.).

Complexes of the invention can provide tools for the characterization of receptors, binding proteins, and other binding sites, and can help elucidate the mechanism of action of the peptide hormones. For example, because the blocking antibodies described herein mimic the receptors to which the peptide hormones bind, the antibodies can serve as surrogate receptors. Thus, small molecule/antibody complexes can serve as artificial ligand/receptor complexes. See also the types of studies described in Poyner *et al.* (2002) *Pharmacol. Rev.* 54, 233-246 and Pio *et al.* (2002) *Microsc. Res. Tech.* 57, 23-27. In another embodiment, a complex between a peptide or antibody and a compound of the invention can be used to identify and/or characterize other compounds that exhibit more effective antagonist of agonist activity, *e.g.*, as discussed above.

When a modulatory compound of the invention is administered to an animal, a complex of the compound and AM or GRP may form *in vivo* (in the animal).

Another aspect of the invention is a kit, suitable for performing any of the methods (*e.g.*, assays) of the invention. For example, the kit may be suitable for treating a subject (*e.g.*, a subject suffering from a condition mediated by aberrant expression and/or activity of AM or GRP), or for detecting an AM or GRP peptide, *in vitro* or *in*

vivo. The components of the kit will vary according to which method is being performed. Generally, a kit of the invention comprises one or more of the compounds of formula I through formula XVII, or a pharmaceutical composition comprising said compound(s) and a pharmaceutically acceptable carrier. The kits also optionally contain means (*e.g.*, suitable reagents) for monitoring disease conditions and/or for detecting AM or GRP. Reagents for performing suitable controls may also be included.

Optionally, the kits comprise instructions for performing the method. Kits of the invention may further comprise a support on which a cell can be propagated (*e.g.*, a tissue culture vessel) or a support to which a reagent used in the method is immobilized. Other optional elements of a kit of the invention include suitable buffers, media components, or the like; a computer or computer-readable medium for storing and/or evaluating the assay results; logical instructions for practicing the methods described herein; logical instructions for analyzing and/or evaluating the assay results as generated by the methods herein; containers; or packaging materials. The reagents of the kit may be in containers in which the reagents are stable, *e.g.*, in lyophilized form or stabilized liquids. The reagents may also be in single use form, *e.g.*, in single dosage form for use as therapeutics, or in single reaction form for diagnostic use.

Kits of the invention have many uses, which will be evident to the skilled worker. For example, they can be used in experiments to study factors involved receptor-mediated activities; to detect the presence of AM or GRP in a cell or tissue, *in vitro* or *in vivo*; to treat a condition mediated by aberrant expression and/or activity of AM or GRP; to monitor the course of such a treatment; or to identify more effective modulatory agents for AM or GRP. A modulatory agent of interest can be characterized by performing assays with the kit, and comparing the results to those obtained with known agents (or by comparison to a reference). Such assays are useful commercially, *e.g.*, in high-throughput drug studies.

BRIEF DESCRIPTION OF THE DRAWINGS

Various other features and attendant advantages of the present invention will be more fully appreciated as the same becomes better understood when considered in conjunction with the accompanying drawings.

FIG. 1 illustrates primary screen (step #1 of the method) using a blocking monoclonal antibody. **Fig. 1A** shows a schematic representation of the primary screening process. **Fig. 1B** shows a photograph of part of a fully developed AM-coated 96-well plate used for the initial screening of the library. Wells A1 and A2 are not coated with AM and provide the value for non-specific background. Wells A3 and A4 have been exposed to all the reagents but the competitors and their color value provides the maximum binding for the assay. Wells A5 and A6 have been exposed to 1.2 μg/ml unlabeled monoclonal antibody and constitute a positive-competition control. Individual small molecules from the library were assayed in duplicates in wells B and C. Wells B10 and C10 contain compound VIII (697165), one of the successful competitors. Wells A7-A12 are empty. Actual absorbance values were quantified in a plate reader.

Figure 2 shows a secondary screen (step #2 of the method) for AM-active compounds. This figure shows secondary screening of promising compounds by induction of intracellular cAMP levels in Rat2 cells (**Figures 2A- 2C**) and in HEK 293 cells transfected with CRLR and RAMP1 (**Figure 2D**). cAMP levels were quantified by radioimmunoassay and are represented as variations from the value of the first bar, arbitrarily expressed as 100. **Figure 2A** shows variations on intracellular cAMP levels induced by a superagonist compound (compound VIII, or 697165) and an antagonist (compound VI, or 79422) in the presence and absence of 100 nM AM. Forskolin was added as a positive control. Asterisks represent statistical significance when compared to the untreated control (first bar) or as indicated by the horizontal bars. **Figure 2B** shows dose-dependent elevation of cAMP induced by the superagonist compound VIII in the presence of 100 nM AM. Asterisks represent statistical significance when compared to addition of AM alone (first bar). **Figure 2C** shows a comparison of the effects elicited by other members of the family of compound VIII in the presence of 100 nM AM. Asterisks represent statistical significance when compared to addition of AM alone (second bar). **Figure 2D** shows the lack of effect of several compounds in the presence of 100 nM CGRP in the activation of the CGRP receptor in HEK 293 cells. Asterisks represent statistical significance when compared to addition of CGRP alone (second bar).

Bars represent mean ± standard deviation of three independent determinations. n.s.: No significant differences; * : $p<0.05$; ** : $p<0.01$; ***: $p<0.001$.

Figure 3 shows a secondary screen for GRP-active compounds. This figure shows an analysis of second messengers for compounds that interfere with GRP binding. **Figure 3A** shows a quantification of IP3 levels in cell line H1299 exposed to different compounds in the presence or absence of 100 nM GRP. Bars represent mean ± standard deviation of three independent determinations. Asterisks represent statistical significance when compared to addition of GRP alone (second bar). n.s.: No significant differences; * : $p<0.05$; ** : $p<0.01$; ***: $p<0.001$. **Figure 3B** shows Ca^{2+} response induced by 1 nM GRP in H1299 cells. **Figure 3C** shows that preincubation of H1299 cells with compound XIV (54671) for 1 min. dramatically reduces the Ca^{2+} response elicited by 1 nM GRP.

Figure 4 shows blood pressure regulation by AM-active compounds. This figure shows representative blood pressure recordings in hypertensive SHR (A,B) and in normotensive Lewis/ssncr (C) rats following the intravenous injection of AM antagonists (XII, or 128911; XIII, or 145425), agonists (I, or 16311), or vehicle (PBS+DMSO). Synthetic AM was added in B for comparison purposes.

Figure 5 shows the antiangiogenic effect of a GRP antagonist. This figure shows cord formation assay in matrigel with bovine retinal microvascular endothelial cells. **Figure 5A** shows a negative control with no additions. **Figure 5B** shows that a complex tubular lattice is induced by 5 nM GRP. **Figure 5C** shows that the simultaneous addition of antagonist compound XV (77427) (0.5 μM) reduces network complexity.

Figure 6 shows the chemical structure of selected AM antagonists (**Fig. 6A**) and superagonists (**Fig. 6B**).

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius; and, unless otherwise indicated, all parts and percentages are by weight.

EXAMPLES

Example 1 - Small molecule library

The small molecule repository that the NCI has collected since 1955 was used. This library contains about 500,000 compounds organized in 2,000 families of chemically similar molecules. The construction of the library has been described in Voigt *et al.* (*supra*) and can be viewed at the web site cactus.nci.nih.gov/ncbidb2. All compounds were provided diluted in DMSO.

Example 2 – Reagents

Synthetic human AM and GRP were purchased from Peninsula (S. Carlos, California). Synthetic CGRP and forskolin were obtained from Sigma (St. Louis, Missouri). Blocking monoclonal antibodies against AM^{30} and GRP^{20} were produced in-house and labeled with peroxidase using EZ-Link Plus Activated Peroxidase (Pierce, Rockford, Illinois).

Example 3 – Primary screening for AM and GRP (step #1 of the assay)

Human synthetic AM was solid-phased into PVC 96-well plates (Fisher Scientific, Pittsburgh, Pennsylvania) by incubating 50 µl of AM (at 1 nmols/µl) per well for 1 h. To solid-phase GRP into the plates, these were previously treated with glutaraldehyde as described (Kasprzyk *et al.* (1988) *Anal. Biochem.* 174, 224-234). After discarding the coating solution, the plates were blocked with 200 µl per well of 1% bovine serum albumin (BSA) in phosphate buffer saline (PBS). After 1 h, this solution was aspirated off and 50 µl containing 1 µM of one of the compounds of the library in PBS was added per well. Immediately after, 50 µl of labeled antibody (at 2.4 µg/ml) were added on each well and the solution was allowed to react for 1 h. Following 3 thorough washes with 1% BSA in PBS to remove the unbound antibodies, peroxidase activity was developed using o-phenylenediamine dihydrochloride (Sigma) as a substrate. The reaction product was quantified with a plate reader (Spectra Rainbow, Tecan, Austria) at 450 nm. Each plate contained several internal controls including wells without any coating that are used to calculate non-specific binding; wells where no

potential antagonists were added, which provided maximum binding; and wells where the unlabeled antibody (at 1.2 μg/ml) substituted the small molecule, as a positive inhibition control (Fig. 1B). Each compound was added to duplicate wells in the same plate. A positive hit was defined as a compound that was able to significantly reduce the amount of reaction product in three independent plates.

Example 4 – Analysis of second messengers (step #2 of the assay)

a. *cAMP analysis for AM and CGRP*

The fibroblast cell line Rat2 has been shown to contain specific AM receptors and react to AM addition by elevating its intracellular cAMP contents. This cell line was obtained from the American Tissue Culture Collection (ATCC, Manassas, Virginia) and maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, California). Cells were seeded in 24-well plates at 2 x 10^4 cells/well and incubated at 37 °C in 5% CO_2 until they reached 80% confluency. Before the assay, cells were incubated for 15 min in TIS medium (RPMI-1640 plus 10 μg/ml transferrin, 10 μg/ml insulin, and 50 nM sodium selenite) containing 1% BSA, 1 mg/ml bacitracin, and 100 μM isobutylmethylxanthine. Peptides and small molecules were applied in the same medium for 5 min at the indicated concentrations in a volume of 250 μl. The reaction was terminated by adding an equal volume of ice-cold ethanol. cAMP contents were measured using the Biotrac cAMP radioimmunoassay (Amersham Biosciences, Piscataway, New Jersey), as described (Pio *et al.* (2001) *J. Biol. Chem.* 276, 12292-12300).

A cell line expressing the CGRP receptor was generated by transfecting HEK 293 cells with CRLR and RAMP1 (a generous gift from Dr Debbie Hay, Hammersmith Hospital, London, UK). The analysis was performed as above, but using CGRP instead of AM as the main agonist. In both cases, forskolin was used as a positive control at 50 μM.

b. *IP_3 and Ca^{2+} analysis for GRP*

The lung cancer cell line H-1299 has been shown to contain specific GRP receptors. This cell line was obtained from ATCC and cultured as the other cell lines.

The signal transduction pathway for GRP includes elevation of intracellular levels of IP_3 and Ca^{2+} and these were investigated as previously shown (Ryan *et al.* (1998) *J. Biol. Chem*, 273, 13613-13624). Briefly, to quantify IP_3, contents cells were subcultured into 24-well plates (5 x 10^4 cells/well). After a 24 h incubation period at 37 °C, the cells were incubated with 3 μCi/ml myo-[^{3}H]inositol in growth medium supplemented with 2% FBS for an additional 24 h. Incubation volumes were 500 μl of assay buffer/well containing 135 mM sodium chloride, 20 mM HEPES (pH 7.4), 2 mM calcium chloride, 1.2 mM magnesium sulfate, 1 mM EGTA, 20 mM lithium chloride, 11.1 mM glucose, and 0.05% BSA (v/v) with or without any of the molecules studied at 37 °C for 30 min. Experiments were terminated with 1 ml of ice-cold hydrochloric acid/methanol (0.1%, v/v). [^{3}H]IP_3 was eluted off Dowex AG-1-X8 anion exchange columns with 2 ml of 1 mM ammonium formate and 100 mM formic acid. Each of the eluates was collected and mixed with 10 ml of scintillation mixture (BioSafe, Research Products International Corp, Mount Prospect, Illinois), and the radioactivity was measured in a LS 3801 β counter (Beckman, Somerset, New Jersey).

Calcium levels were analyzed by loading the cells with 2 μM FURA-2/AM (Molecular Probes, Eugene, Oregon) for 30 min at 37 °C. After washing two times with TIS, 2 ml of cell suspension were placed in a Delta PTI Scan 1 spectrofluorimeter (Photon Technology International, South Brunswick, New Jersey) equipped with a stir bar and water bath (37 °C). Fluorescence was measured at dual excitation wavelengths of 340 nm and 380 nm using an emission wavelength of 510 nm.

Example 5 – Measurement of blood pressure *in vivo*

AM is a potent and long-lasting vasodilator. Therefore it was expecteded that AM antagonists would elevate blood pressure and AM superagonists would decrease it further. In consequence, suspected antagonists were analyzed in normotensive rats (10-week-old Lewis/ssncr males, SAIC, Frederick, Maryland) and suspected superagonists in hypertensive animals (10-week-old SHR males, Taconic Farms, Germantown, NY).

Animals were anesthetized with 3% halothane, intubated, and maintained with 1% halothane in 70% nitrous oxide and 30% oxygen (VMS Anesthesia Machine, Matrx, Medical Inc., Orchard Park, New York) at 82 strokes/min. Rectal temperature was

monitored through the experiment. A PE50 catheter was placed on the right femoral artery and arterial blood pressure was recorded through a P23XL transducer (Grass Instruments, Quincy, Massachusetts). Peptides and small molecules were injected into the right femoral vein through another catheter. All procedures were performed under a protocol approved by the National Institutes of Health.

Example 6 - Cord formation assay

Formation of tube-like structures was performed as described in Kubota *et al.* (1988) *J. Cell Biol.* 107, 1589-1598 and Nam *et al.* (2003) *Phytother. Res.* 17, 107-111. Briefly, a thin layer of Matrigel (Collaborative Biomedical Products, Bedford, Massachusetts) was allowed to polymerize at the bottom of 24-well plates. Bovine retinal microvascular endothelial cells (a gift from Dr Patricia Becerra, NEI, NIH) were resuspended in Human Endothelial-SFM Basal Growth Medium (Invitrogen) and applied to triplicate wells (2×10^5 cells/500 µl medium) in the presence or absence of the test compounds. After an overnight incubation at 37 °C, the tubular structures were photographed (3 pictures per well at 10x) and the number of knots per photographic field were counted as a measure of lattice complexity.

Example 7 - Statistics

Different treatments were compared with two-tailed Student's *t* test. P values smaller than 0.05 were considered statistically significant.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make changes and modifications of the invention to adapt it to various usage and conditions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The preceding preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The entire disclosure of all applications, patents and publications, cited above and in the figures are hereby incorporated by reference.

References

US patent application 20020019347; US patent application 20020055615 (Cuttitta *et al.*); Isumi *et al.* (1998) *Endocrinology* 139, 2552-2563; Coppock *et al.* (1999) *Biochem J.* 338, 15-22; Ishizaka *et al.* (1994) *Biochem. Biophys. Res. Comm.* 200, 642-646; Heimbrook *et al.* (1991) *J. Med. Chem.* 34, 2102-2107; USP 5,834,433; USP 5,047,502; Draoui, Dissertation, George Washington University, 1993

WE CLAIM:

1. A complex, comprising a compound of formula I to formula XIII, in association with an adrenomedullin (AM) peptide.

2. The complex of claim 1, which is in an animal.

3. The complex of claim 1, which is *in vitro*.

4. A complex, comprising a compound of formula XIV to formula XVII, in association with a gastric releasing peptide (GRP).

5. The complex of claim 4, which is in an animal (*e.g.*, a mammal).

6. The complex of claim 4, which is *in vitro*.

7. A pharmaceutical composition, comprising a compound of formula I to formula XVII and a pharmaceutically acceptable carrier.

8. A method for inhibiting an activity of an AM peptide, comprising contacting the peptide with an effective amount of a compound of formula I, II, III, IV, V, VI or VII.

9. The method of claim 8, wherein the peptide and compound are in an animal.

10. The method of claim 8, wherein the peptide and compound are *in vitro*.

11. The method of claim 8, wherein the activity of the AM peptide is stimulation of the level of intracellular cAMP.

12. The method of claim 8, wherein the activity of the AM peptide is vasodilation.

13. A method for treating a condition that is mediated by over-expression and/or activity of AM, comprising administering to a patient in need of such treatment an effective amount of a compound of formula I, II, III, IV, V, VI or VII.

14. The method of claim 13, wherein the condition is type 2 diabetes or cancer.

15. A method for stimulating an activity of an AM peptide, comprising contact the peptide with an effective amount of a compound of formula VIII, IX, X, XI, XII or XIII.

16. The method of claim 15, wherein the peptide and compound are in an animal.

17. The method of claim 15, wherein the peptide and compound are *in vitro*.

18. The method of claim 15, wherein the activity of the AM peptide is stimulating the level of intracellular cAMP.

19. The method of claim 15, wherein the activity of the AM peptide is vasodilation

20. A method for treating a condition that is mediated by under-expression and/or activity of AM, comprising administering to a patient in need of such treatment an effective amount of a compound of formula VIII, IX, X, XI, XII or XIII.

21. The method of claim 20, wherein the condition is renal or cardiovascular disease, sepsis, or central nervous system ischemia.

22. A method for inhibiting an activity of a GRP peptide, comprising contacting the peptide with an effective amount of a compound of formula XIV, XV or XVI.

23. The method of claim 22, wherein the peptide and compound are in an animal.

24. The method of claim 22, wherein the peptide and compound are *in vitro*.

25. The method of claim 22, wherein the activity of the GRP peptide is the stimulation of intracellular IP_3 or Ca^{+2}.

26. The method of claim 22, wherein the activity of GRP is stimulating angiogenesis, suppressing food intake, regulating glucose homeostasis, or stimulating hypotension.

27. A method for treating a condition that is mediated by over-expression and/or activity of GRP, comprising administering to a patient in need of such treatment an effective amount of a compound of formula XIV, XV or XVI.

28. The method of claim 27, which is a method for reducing tumor growth, or treating low blood pressure or an eating disorder.

29. A method for stimulating an activity of a GRP peptide, comprising contacting the peptide with an effective amount of a compound of formula XVII.

30. The method of claim 29, wherein the peptide and compound are in an animal.

31. The method of claim 29, wherein the peptide and compound are *in vitro*.

32. The method of claim 29, wherein the activity of the GRP peptide is the stimulation of intracellular IP_3 or Ca^{+2}.

33. The method of claim 29, wherein the activity of GRP is stimulating angiogenesis, suppressing food intake, regulating glucose homeostasis, or stimulating hypotension.

34. A method for treating a condition that is mediated by under-expression and/or activity of GRP, and/or that would benefit from increased expression of GRP comprising administering to a patient in need of such treatment an effective amount of a compound of formula XVII.

35. The method of claim 34, wherein in the condition is obesity, diabetes, hypertension, coronary or peripheral artery disease, tissue ischemia, organ or tissue transplantation, or acceleration or enhancing of fracture repair or wound healing.

36. A method for detecting an AM peptide, comprising

contacting a sample suspected of containing the peptide with one or more detectably labeled compounds of formula I through Formula XIII, and

detecting labeled compound that is associated with the peptide.

37. A method for detecting a GRP peptide, comprising

contacting a sample suspected of comprising the peptide with one or more detectably labeled compounds of formula XIV through Formula XVII, and

detecting labeled compound that is associated with the peptide.

38. The method of claim 36 or 37, which is performed *in vivo*.

39. The method of claim 36 or 37, which is performed *in vitro*.

40. A kit suitable for treating a subject suffering from a condition mediated by aberrant expression and/or activity of adrenomedullin (AM), comprising one or more of the compounds of formula I to formula XIII, or a pharmaceutical composition comprising said compound(s) and a pharmaceutically acceptable carrier, and, optionally, a container or packaging material.

41. A kit suitable for treating a subject suffering from a condition mediated by an aberrant expression and/or activity of gastrin releasing peptide (GRP), comprising one or more of the compounds of formula XIV to formula XVII, or a pharmaceutical composition comprising said compound(s) and a pharmaceutically acceptable carrier, and, optionally, a container or packaging material.

42. A kit suitable for detecting an AM peptide, comprising

a) one or more of the compounds of formula I to formula XIII, which is detectably labeled, and, optionally,

b) means to detect the labeled compound associated with (bound to) the peptide.

43. A kit suitable for detecting a GRP peptide, comprising

a) one or more of the compounds of formula XIV to formula XVII, which is detectably labeled, and, optionally,

b) means to detect the labeled compound associated with (bound to) the peptide.

44. A kit of claim 40 or 41, which is suitable *in vivo* detection, further comprising a pharmaceutically acceptable carrier.

ABSTRACT OF THE INVENTION

This invention relates, *e.g.*, to methods for inhibiting or stimulating an activity of an adrenomedullin (AM) or gastrin releasing peptide (GRP) peptide hormone, comprising contacting the peptide with a small molecule, non-peptide, modulatory agent of the invention. Complexes of these modulatory agents with other components, such as the peptides or blocking antibodies specific for the peptides, are also described, as are pharmaceutical compositions comprising the modulatory agents, and methods for using the modulatory agents to diagnose or treat patients.

::ODMA\PCDOCS\DC2DOCS1\483353\1

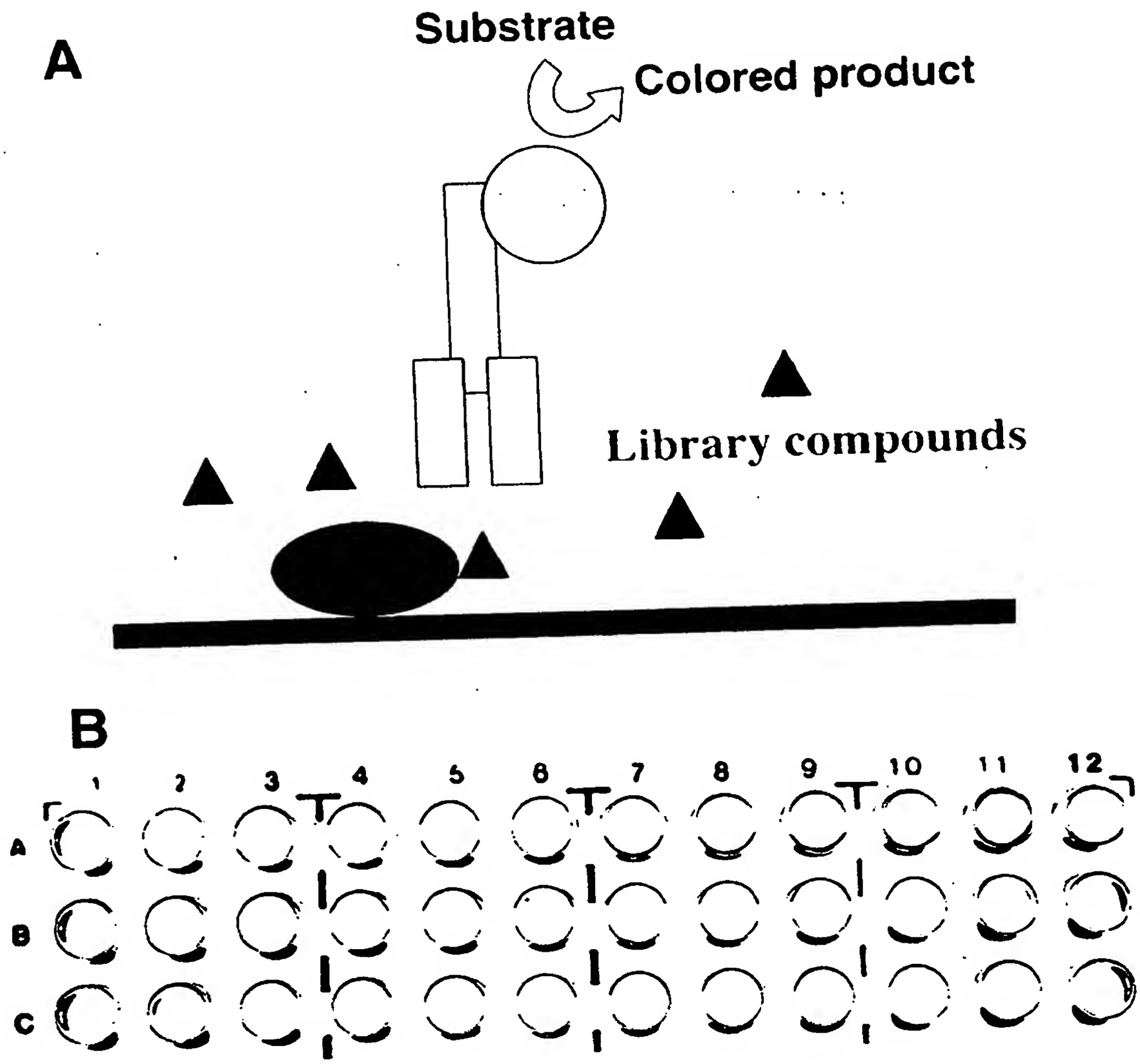

A
Substrate
Colored product
Library compounds
B
1
2
3
4
5
6
7
8
9
10
11
12
A
B
C

Figure 1

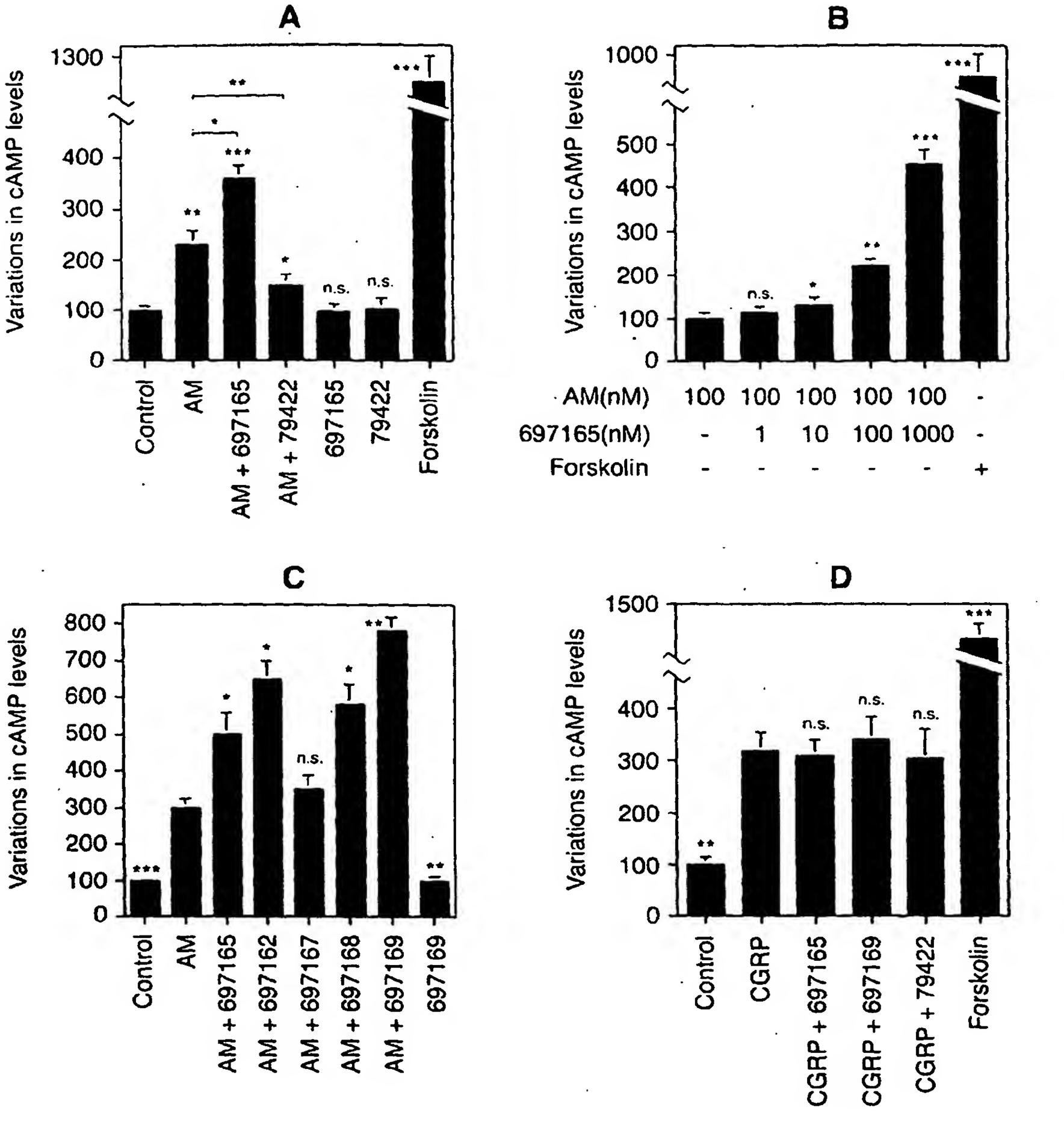

A
Variations in cAMP levels
1300
400
300
200
100
0
Control
AM
AM + 697165
AM + 79422
697165
79422
Forskolin
n.s.
n.s.
B
Variations in cAMP levels
1000
500
400
300
200
100
0
n.s.
AM(nM) 100 100 100 100 100 -
697165(nM) - 1 10 100 1000 -
Forskolin - - - - - +
C
Variations in cAMP levels
800
700
600
500
400
300
200
100
0
Control
AM
AM + 697165
AM + 697162
AM + 697167
AM + 697168
AM + 697169
697169
n.s.
D
Variations in cAMP levels
1500
400
300
200
100
0
Control
CGRP
CGRP + 697165
CGRP + 697169
CGRP + 79422
Forskolin
n.s.
n.s.
n.s.

Figure 2

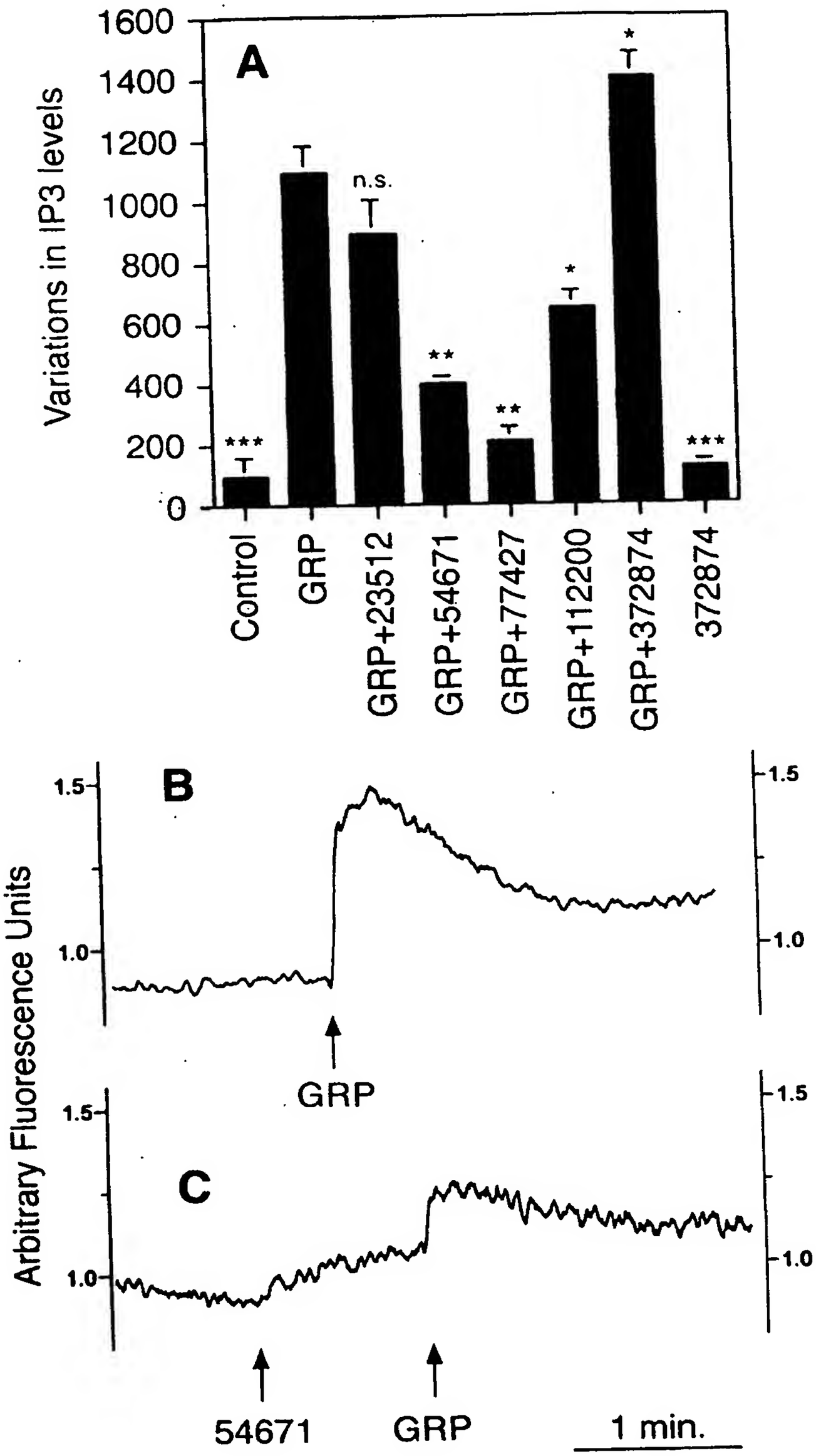

A
Variations in IP3 levels
1600
1400
1200
1000
800
600
400
200
0
***
n.s.
**
**
*
*
***
Control
GRP
GRP+23512
GRP+54671
GRP+77427
GRP+112200
GRP+372874
372874
B
1.5
1.0
GRP
C
Arbitrary Fluorescence Units
54671
GRP
1 min.

Figure 3

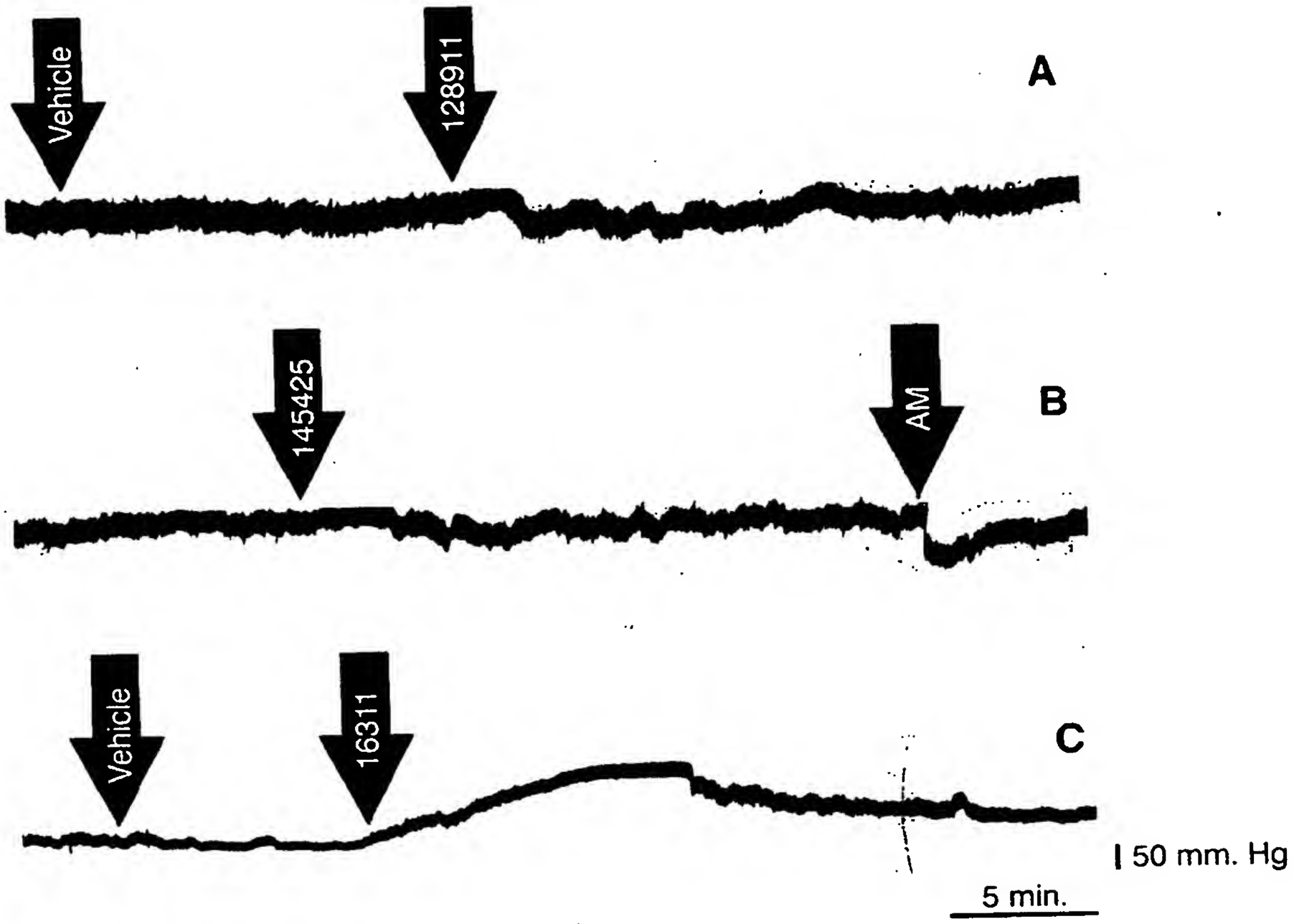


Figure 4

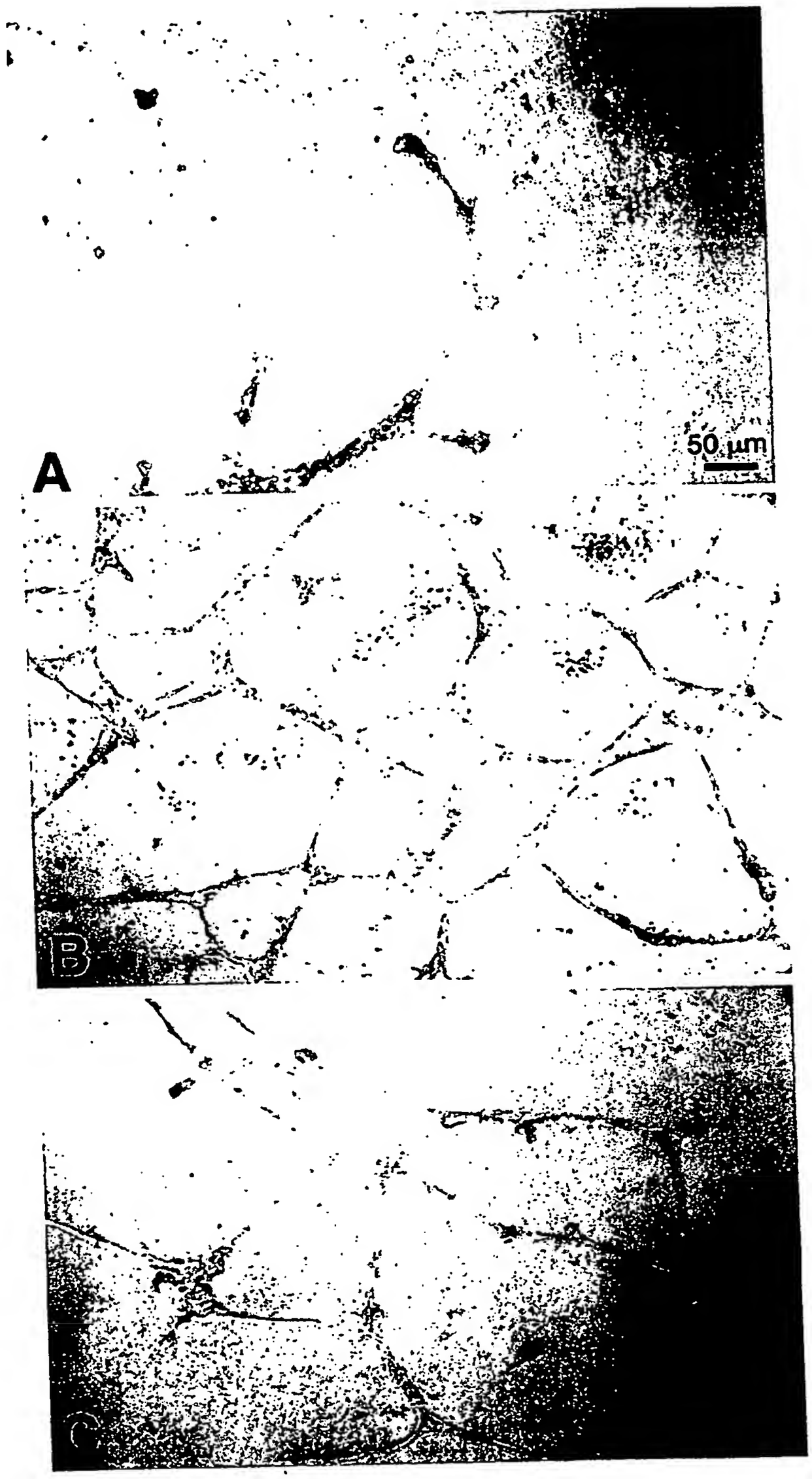
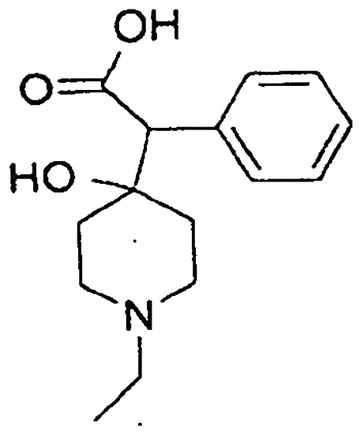
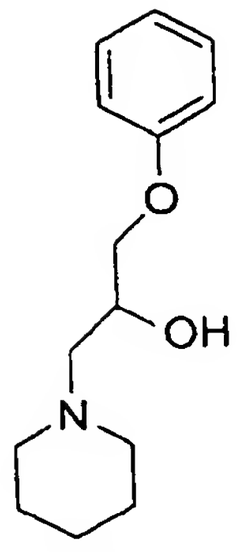


Figure 5

A



16311



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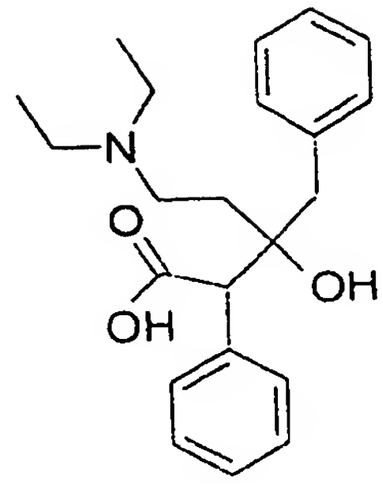

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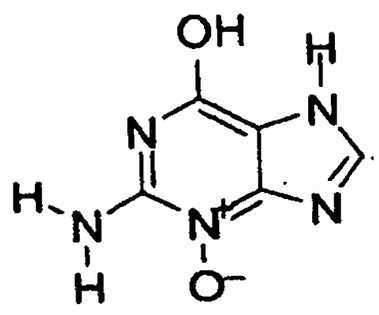
B



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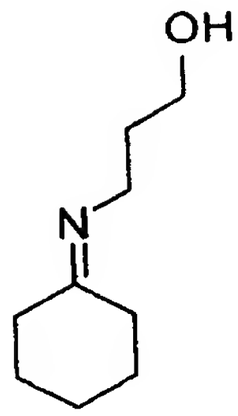
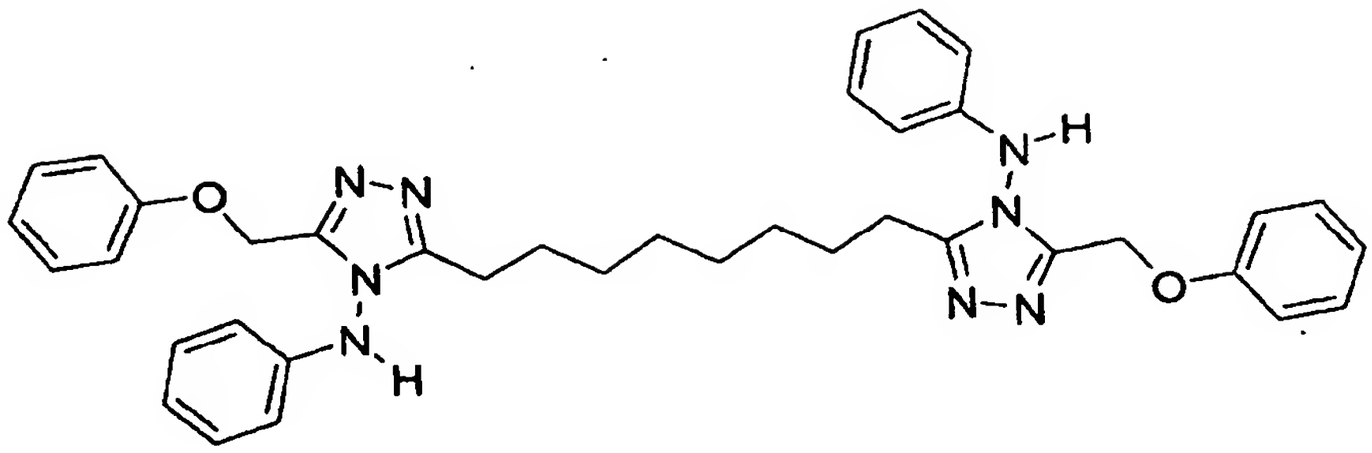


128911



697165

Figure 6

APPLICATION DATA SHEET

Application Information

Application Number::	Not yet assigned
Filing Date::	September 8, 2003
Application Type::	Provisional
Subject Matter::	Utility
Suggested Classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	
Number of CD Disks::	
Number of Copies of CDs::	
Sequence Submission?::	
Computer Readable Form (CFR)?::	
Number of Copies of CFR::	
Title::	NON PEPTIDE AGONISTS AND ANTAGONISTS OF ADRENOMEDULLIN AND GASTRIC RELEASING PEPTIDE
Attorney Docket Number::	31978-192619
Request for Early Publication?::	
Request for Non-Publication?::	
Suggested Drawing Figure::	
Total Drawing Sheets::	1
Small Entity?::	NO
Latin Name::	
Variety Denomination Name::	
Petition Included?::	
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.::	

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship::	USA
Country::	USA
Status::	Full Capacity
Given Name::	Frank
Middle Name::	
Family Name::	CUTTITTA
Name Suffix::	
City of Residence::	Bethesda
State or Province of Residence::	Maryland
Country of Residence::	USA
Street of Mailing Address::	Building 10, Room 13N 262
City of Mailing Address::	Bethesda
State or Province of Mailing Address::	Maryland
Country of Mailing Address::	USA
Postal or Zip Code of Mailing Address::	20892

Applicant Authority Type::	Inventor
Primary Citizenship::	Spain
Country::	Spain
Status::	Full Capacity
Given Name::	Alfredo
Middle Name::	
Family Name::	MARTINEZ
Name Suffix::	
City of Residence::	Bethesda
State or Province of Residence::	Maryland
Country of Residence::	USA
Street of Mailing Address::	5225 Pooks Hill Road, Apr. 1614S

City of Mailing Address::	Bethesda
State or Province of Mailing Addr ss::	Maryland
Country of Mailing Address::	USA
Postal or Zip Code of Mailing Address::	20814

Applicant Authority Type::	Inventor
Primary Citizenship::	
Country::	
Status::	Full Capacity
Given Name::	
Middle Name::	
Family Name::	
Name Suffix::	
City of Residence::	
State or Province of Residence::	
Country of Residence::	
Street of Mailing Address::	
City of Mailing Address::	
State or Province of Mailing Address::	
Country of Mailing Address::	
Postal or Zip Code of Mailing Address::	

Applicant Authority Type::	Inventor
Primary Citizenship::	
Country::	
Status::	
Given Name::	
Middle Name::	
Family Name::	

Name Suffix::

City of Residenc ::

State or Province of Residence::

Country of Residence::

Street of Mailing Address::

City of Mailing Address::

State or Province of Mailing Address::

Country of Mailing Address::

Postal or Zip Code of Mailing Address::

Correspondence Information

Correspondence Customer Number:: 26694

Phone Number::

Fax Number::

E-Mail Address::

Representative Information

Representative Customer Number:: 26694

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
	Continuation of		
	Continuation of		
	Continuation of		
	Continuation of		

Foreign Priority Information

Country::	Application Number::	Filing Date::	Priority Claimed::

Assignee Information

Assignee Name::	National Institutes of Health
Street of Mailing Address::	Office of Technology Transfer 6011 Executive Boulevard
City of Mailing Address::	Rockville,
State or Province of Mailing Address::	MD
Country of Mailing Address::	USA
Postal or Zip Code of Mailing Address::	20852

APPENDIX

Structures of Some of The Compounds of The invention.

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

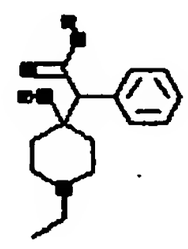
Fields: NSC Number, Molecular Weight, Name (ACD) — Retrieve

Visualization: GIF Image Gallery — Display

Miscellaneous: Restart Query (at first record) — Execute

Structure

16311



	NSC Number	Formula	CAS	#Names	Sample Name
☑	16311	$C_{15}H_{21}NO_3$	5449-34-3	1	(1-ethyl-4-hydroxy-4-piperidinyl)(phenyl)acetic acid

Invert Selection

Date: 2003-08-25 20:14:07

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

Fields: NSC Number, Molecular Weight, Name (ACD)

Retrieve

Visualization: GIF Image Gallery

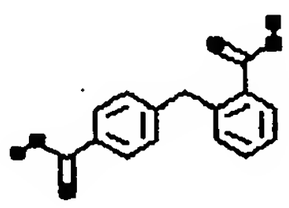
Display

Miscellaneous: Restart Query (at first record)

Execute

Structure

37133



	NSC Number	Formula	CAS	#Names	Sample Name
☑	37133	$C_{15}H_{12}O_4$	6268-08-2	0	*No Name*

Invert Selection

Date: 2003-08-25 20:13:36

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

Fields: NSC Number, Molecular Weight, Name (ACD)

Retrieve

Visualization: GIF Image Gallery

Display

Miscellaneous: Restart Query (at first record)

Execute

Structure

48747

NSC Number	Formula	CAS	#Names	Sample Name
☑ 48747	$C_{11}H_{10}N_2O_3S$	4602-47-5	1	5-((benzyloxy)methyl)-1,2,3-thiadiazole-4-carboxylic acid

Invert Selection

Date: 2003-08-25 20:13:12

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

Fields: NSC Number, Molecular Weight, Name (ACD) — Retrieve

Visualization: GIF Image Gallery — Display

Miscellaneous: Restart Query (at first record) — Execute

Structure

28086

NSC Number	Formula	CAS	#Names	Sample Name
☑ 28086	$C_{26}H_{20}O_3$	6334-91-4	1	di[1,1'-biphenyl]-4-yl(hydroxy)acetic acid

Invert Selection

Date: 2003-08-25 20:12:47

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

Fields: NSC Number, Molecular Weight, Name (ACD)

Retrieve

Visualization: GIF Image Gallery

Display

Miscellaneous: Restart Query (at first record)

Execute

Structure

79422

	NSC Number	Formula	CAS	#Names	Sample Name
☑	79422	$C_8H_{15}NO_3S$	*(None)*	1	1-azabicyclo[2.2.2]oct-3-yl methanesulfonate

Invert Selection

Date: 2003-08-25 20:12:21

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile
Output in 3D ☐ Strip H ☐
Max #Records: 100

Fields: NSC Number
Molecular Weight
Name (ACD)

Retrieve

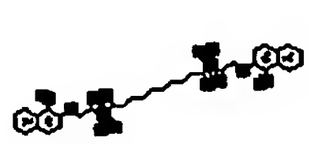
Visualization: GIF Image Gallery

Display

Miscellaneous: Restart Query (at first record)

Execute

Structure
697162



IX

NSC Number	Formula	CAS	#Names	Sample Name
☑ 697162	$C_{34}H_{38}N_8O_4$ *(None)*		1	2-((4-amino-5-(8-(4-amino-5-(((1-hydroxy-2-naphthyl)oxy) methyl)-4H-1,2,4-triazol-3-yl)octyl)-4H-1,2,4-triazol-3-yl) methoxy)-1-naphthol

Invert Selection

Date: 2003-08-25 20:11:58

Operations with this Dataset of 1 Structure:

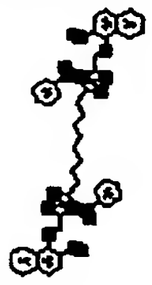
Data Retrieval: Format: SDFile
Output in 3D ☐ Strip H ☐
Max #Records: 100
Fields: NSC Number, Molecular Weight, Name (ACD) — Retrieve

Visualization: GIF Image Gallery — Display

Miscellaneous: Restart Query (at first record) — Execute

Structure

697168



NSC Number	Formula	CAS	#Names	Sample Name
☑ 697168	$C_{46}H_{46}N_8O_4$ *(None)*		1	1-((4-anilino-5-(8-(4-anilino-5-(((2-hydroxy-1-naphthyl)oxy methyl)-4H-1,2,4-triazol-3-yl)octyl)-4H-1,2,4-triazol-3-yl) methoxy)-2-naphthol

Invert Selection

Date: 2003-08-25 20:11:36

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile
Output in 3D ☐ Strip H ☐
Max #Records: 100

Fields: NSC Number / Molecular Weight / Name (ACD)

Retrieve

Visualization: GIF Image Gallery

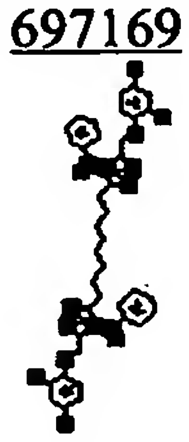
Display

Miscellaneous: Restart Query (at first record)

Execute

Structure

697169



XI

NSC Number	Formula	CAS	#Names	Sample Name
☑ 697169	$C_{38}H_{38}Cl_4N_8O_2$	*(None)*	1	N-(3-(8-(4-anilino-5-((2,4-dichlorophenoxy)methyl)-4H-1,2,4-triazol-3-yl)octyl)-5-((2,4-dichlorophenoxy)methyl-4H-1,2,4-triazol-4-yl)-N-phenylamine

Invert Selection

Date: 2003-08-25 20:11:15

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

Fields: NSC Number, Molecular Weight, Name (ACD) — Retrieve

Visualization: GIF Image Gallery — Display

Miscellaneous: Restart Query (at first record) — Execute

Structure

54671

XIV

	NSC Number	Formula	CAS	#Names	Sample Name
☑	54671	$C_6H_8N_4O_4$	7403-61-4	2	Sydnone, 3, 3'-ethylenedi-

Invert Selection

Date: 2003-08-25 20:10:34

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

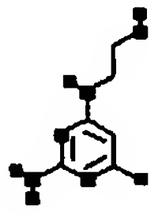
Fields: NSC Number, Molecular Weight, Name (ACD) — Retrieve

Visualization: GIF Image Gallery — Display

Miscellaneous: Restart Query (at first record) — Execute

Structure

77427



XV

	NSC Number	Formula	CAS	#Names	Sample Name
☑	77427	$C_6H_9ClN_4O$	2846-77-7	1	2-((2-amino-6-chloro-4-pyrimidinyl)amino)ethanol

Invert Selection

Date: 2003-08-25 20:10:14

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

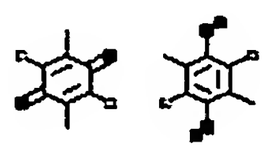
Fields: NSC Number, Molecular Weight, Name (ACD) — Retrieve

Visualization: GIF Image Gallery — Display

Miscellaneous: Restart Query (at first record) — Execute

Structure

112200



XVI

NSC Number	Formula	CAS	#Names	Sample Name
☑ 112200	$C_{16}H_{14}Br_4O_4$ *(None)*		1	2,5-dibromo-3,6-dimethylbenzo-1,4-quinone compound wit 2,5-dibromo-3,6-dimethyl-1,4-benzenediol (1:1)

Invert Selection

Date: 2003-08-25 20:09:49

Operations with this Dataset of 1 Structure:

Data Retrieval: Format: SDFile | Output in 3D ☐ Strip H ☐ | Max #Records: 100

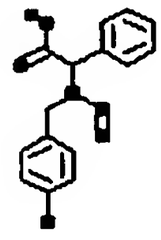
Fields: NSC Number, Molecular Weight, Name (ACD) — Retrieve

Visualization: GIF Image Gallery — Display

Miscellaneous: Restart Query (at first record) — Execute

Structure

372874



XVII

NSC Number	Formula	CAS	#Names	Sample Name
☑ 372874	$C_{15}H_{13}ClN_2O_3$	85152-74-5	1	(1-(4-chlorobenzyl)-2-oxohydrazino)(phenyl)acetic acid

Invert Selection

Date: 2003-08-25 19:27:23

Document made available under the Patent Cooperation Treaty (PCT)

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Number: 60/500,650
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Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

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